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13. ABSTRACT ***(Maximum 200 Words)***

STAT3 has been demonstrated to be an oncogene. To address the STAT3 function in cell growth and cell survival, we have disrupted the STAT3 gene in mice specifically in hepatocytes, endothelial cells or dendritic cells using a bacteriophage Cre/loxP system. We have induced STAT3-deletion exclusively in these cells by crossing a STAT3-floxed mouse with a transgenic mouse expressing Cre under the control of transthyretin, Tie-2 and CD11b promoters, respectively. No specific phenotypes have been identified in STAT3 null cells yet. In addition, to analyze the downstream gene regulation by STAT, we have established representational difference analysis (RDA). We have analyzed the gene regulation in B lymphocytes upon B-cell receptor cross-linking. Furthermore, we have started DNA microarray analysis. Moreover, to address the Task3 (Month 25 - 36), we examined STAT activation in response to neuregulin and platelet-derived growth factor (PDGF) in NIH 3T3 cells. However, we did not find clear correlations between STAT activation and cell growth. Based on these findings, we are planning to further investigate the STAT function in cell growth regulation focusing on analysis of the downstream gene regulation.

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INTRODUCTION:

We have demonstrated that EGF activates STAT1 in some specific cell lines, causing cell growth arrest (9) and apoptosis (8). On the other hand, STAT3 has been shown to be an oncogene (4) and facilitate cell cycle progression (14) and cell survival (24, 26). Furthermore, STAT3 but not STAT1 is activated constitutively in several breast cancer cell lines and further enhanced in response to EGF (15, 30). Therefore, it is very interesting to look into the functional relationship between STAT1 and STAT3 in cancer pathogenesis. To address this issue, we first focused on analyzing STAT3 function using a gene knockout technology in mice. Since conventional STAT3 knockout mice led to embryonic lethality (27), we employed a bacteriophage P1 Cre/loxP system to induce gene disruption in a tissue-specific manner (16). In the last annual report, we reported the generation of a STAT3-floxed mouse, and proposed to disrupt the STAT3 gene specifically in mammary epithelium by crossing the STAT3-floxed mouse with a transgenic mouse that expresses Cre under the control of mouse mammary tumor virus (MMTV) long term repeat (LTR). However, this experiment has been performed and recently published by another group (6). They reported that STAT3 was essential for mammary epithelial apoptosis and normal involution (6), indicating that STAT3 has opposite functions between normal mammary epithelium and breast cancer cells. It suggests that STAT3 may undergo a functional transition from an apoptosis stimulator to an apoptosis inhibitor upon malignant transformation. In addition to breast cancer cells, we have observed constitutive STAT3 activation in human hepatoma cells (data not shown), in which STAT3 has been found important for cell survival (7). To determine if STAT3 has a different function in normal hepatocytes, we have generated liver-specific Cre transgenic mouse lines using a transthyretin promoter (31). Using the transgenic mice, we disrupted the STAT3 gene exclusively in liver, and analyses are ongoing. We have also knocked out STAT3 specifically in endothelial cells and dendritic cells using Cre transgenic mice driven by Tie-2 (13) and CD11b (1, 12) promoters, respectively. Analyses are in the process. In addition, to analyze the downstream gene regulation by STAT, we have established representational difference analysis (RDA). We have analyzed the gene regulation in B lymphocytes upon B-cell receptor cross-linking (22). Furthermore, we have started DNA microarray analysis. Moreover, to address the Task3 (Month 25 – 36), we examined STAT activation in response to neuregulin and platelet-derived growth factor (PDGF) in NIH 3T3 cells. However, we did not find clear correlations between STAT activation and cell growth. Based on these findings, we are planning to further investigate the STAT function in cell growth regulation focusing on analysis of the downstream gene regulation.

BODY:

Liver-specific disruption of STAT3 gene

To elicit targeted expression of Cre in liver, we used transcriptional regulatory signals from the liver specific gene that encodes transthyretin (TTR), a serum thyroid hormone or vitamin A carrier produced in hepatocytes (32). The TTR-Cre transgene was generated by inserting the Cre coding sequence into the second exon of a TTR expression vector previously used to drive transgene expression in liver (31) (Fig. 1). The plasmid was digested with HindIII, and the TTR-Cre transgene was purified by agarose gel electrophoresis and injected into pronuclei of fertilized eggs to generate the TTR-Cre transgenic lines. Six TTR-Cre transgenic founders were identified by PCR. To examine the efficiency and tissue-specificity of Cre-dependent deletion, TTR-Cre transgenic mice were crossed with mice carrying a DNA polymerase β gene (polβ) in a floxed ($pol\beta^F$) germline configuration. DNA from adult offspring was subjected to Southern blot analyses using a polβ-specific probe (Fig. 2A) (16). Deletion of the floxed DNA segment results in removal of the promoter and first exon of polβ, leading to detection of a 3.5-kb band by probe polβ. As expected, one line (TTR19-1) showed a liver-specific recombination of the *loxP* sites with a deletion efficiency of 50 % (Fig. 2B). Such mosaic expression in adult liver was also observed in p53 dominant-negative mutant-transgenic mice generated with the TTR promoter (3). Another line, TTR10-3, directed deletion of the *loxP* sites in pancreas, gut, and gallbladder in addition to liver, with efficiencies of 100 %, 50 %, 50 %, and 95 %, respectively (Fig. 2C). TTR has recently been shown to be expressed in pancreas (2) (Van Dyke, unpublished), and is expressed in fetal gut (21, 25). Expression of the pTTRExV3 vector is often sustained in adult gut (32). Low levels of deletion were also observed in other tissues such as testis.

We have crossed the TTR19-1 mouse with the STAT3-floxed mouse reported last year to induce STAT3 gene inactivation exclusively in hepatocytes. We have examined the effects of STAT3 deficiency on liver development and liver regeneration. However, we have not found any phenotypes specific for STAT3 null liver. Further analyses are ongoing both *in vivo* and *in vitro*.

Endothelial cell- and dendritic cell-specific STAT3 disruption

We knocked out STAT3 specifically in endothelial cells and dendritic cells using Cre transgenic mice driven by Tie-2 (13) and CD11b (1, 12) promoters, respectively. No phenotypes have been identified specific for STAT3 deficiency.

Representational difference analysis of gene regulation in B cell lymphoma cells upon B cell receptor cross-linking

The Burkitt's lymphoma B cells are considered as B cells isolated at a point of differentiation by transformation (5). The Burkitt's lymphoma cell line, BL2, shows cell surface marker proteins characteristics of centroblastic B cell phenotypes which suggests it is of centroblastic B cell origin (11). BCR activation is a particularly important step for late stages of differentiation of B cell. In this paper, we characterized altered gene expression in BL2 cells by BCR cross-linking as a model study of antigen stimulated germinal center centroblastic B cells by applying PCR-coupled cDNA Representational Difference Analysis (RDA). Our results may identify important signaling pathway that exist in complex B, T, FDC, DC networks in the microenvironment of secondary lymphoid organs.

RDA technology is a powerful and efficient technology using PCR amplification with specific linkers as primers combined with subtraction of product of control cells from product of test cells that allows detection of differentially expressed genes (10, 18, 20, 33). A summary of 30 clones identified by RDA analysis of activated BL2 cells is presented in Table 1. We detected gene expression of MDC, IL6R as well as SIRPa, adhesion molecule LFA1, anti-apoptotic A-20, signal regulatory SLP76 and BCAR3, DNA binding proteins EGR2 and DEC1 in BCR cross-linked Burkitt's Lymphoma BL2 cell. It is known that MDC in unstimulated B cell lineage (23) and IL6R in BL2 cell line are not expressed (19, 29). LFA1 molecule is significantly up-regulated upon B cell stimulation with anti-IgM and IL-4 (28). Consistent with previous observations, we detected expression of MDC, IL6R and LFA1 in the BL2 cell stimulated by BCR cross-linking.
We are planning to use this technology to profile gene expression in small pieces of cancer tissues.

DNA microarray analysis

To analyze the gene regulation in cancer cells, we have also started DNA microarray analysis in collaboration with Dr. Howard Jacob in the Human Molecular Genetics Center at the Medical College of Wisconsin where my new laboratory is located. They have established the microarray analysis with rat genes. We are in the process of developing the human microarrays.

Cell growth control by the receptor tyrosine kinase-STAT signaling pathways

We have examined STAT activation in NIH3T3 cells transformed with erbB receptors. The cells were transformed with either erbB2 and erbB4 (NIH3T3/erbB2/4) or erbB2 and erbB3 (NIH3T3/erbB2/3), and stimulated by neuregulin. In NIH3T3/erbB2/4 cells, neuregulin activated STAT1 strongly but STAT3 and STAT5 slightly. In NIH3T3/erbB2/3 cells, neuregulin activated STAT1, STAT3 and STAT5 at the same level. We did not find a correlation between STAT3 activation and erbB-induced transformation or growth of NIH3T3 cells.

To determine if STAT3 is essential for NIH3T3 cell growth in response to PDGF, we established NIH3T3 transformants with dominant-negative forms of STAT3. We used two types of dominant negative STAT3 mutants working in different manners. One is STAT3F, and the other is STAT3AA. STAT3F can't be activated in cells because of the substitution of Y^{705} with F whose phosphorylation is required for STAT3 activation (14). STAT3AA has a mutation in the DNA binding domain (from 432VTEEL436 to VTAAL) which interferes with its DNA-binding (17). Neither of these mutants affected cell growth stimulation by PDGF when evaluated by thymidine incorporation assay. Therefore, we concluded that PDGF stimulated NIH3T3 fibroblast cell growth in a STAT3-independent fashion.

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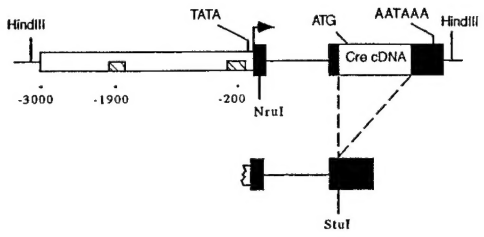

Figure 1. Structure of the transthyretin (TTR)-Cre transgene. pTTRExV3, the transgene expression vector, contains a TTR minigene consisting of 3.0 Kb upstream regulatory region (open box with two striped boxes [liver-specific enhancer]) of the RNA start site, the first exon (black box) and intron, and a partial second exon, followed by an SV 40 polyadenylation sequence (gray box). A 1.1 Kb XhoI-HindIII fragment from plasmid pBS185 carrying the Cre cDNA was blunt-ended, and inserted into the StuI site of the pTTRExV3 vector which contains a TTR minigene consisting of 3.0 kb upstream regulatory region of the RNA start site, the first exon and intron, and a partial second exons (Wu et al., 1996).

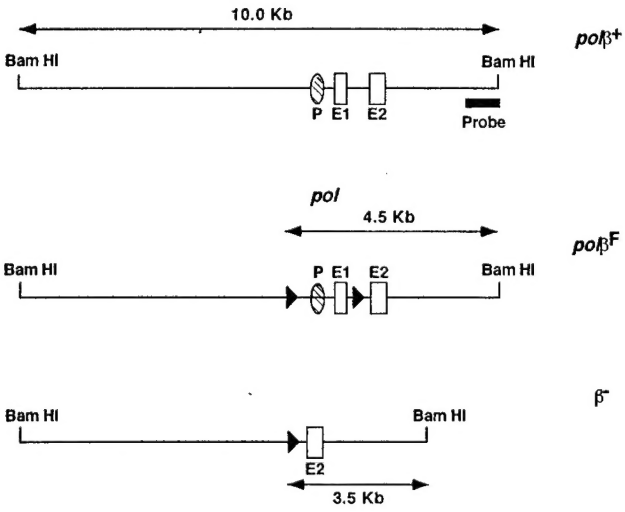

Figure 2A. Polymeraseβ (polβ) locus with the *loxP*-flanked mutation. The promoter (striped oval) and the first exon (open box) are flanked by two *loxP* sequences (triangle) in a polβ^F allele. This *loxP*-flanked region is deleted in a Cre recombinase-dependent manner (polβ$^-$). After digestion of the genome with BamHI, the polβ probe (bold bar) detects 10.0-Kb, 4.5-Kb, and 3.5-Kb fragments of the wild-type (polβ$^+$), polβ^F, and polβ$^-$ allele, respectively, on Southern blot analysis.

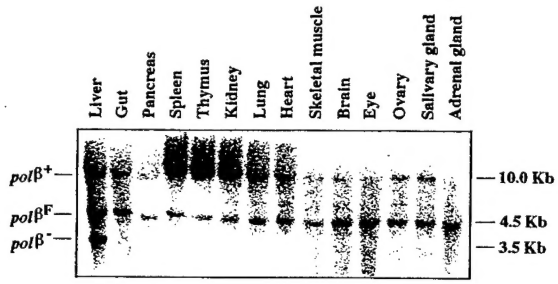

Figure 2B. Southern blot analysis of various tissues from a TTR19-1 mouse carrying polβ$^{F/+}$ alleles. Ten micrograms of genomic DNA from each tissue was digested with BamHI and subjected to Southern blot analysis using the polβ probe. Cre-dependent deletion of the *loxP* sites was observed exclusively in liver with an efficiency of 50 %. No deletion was detected in gallbladder (data not shown).

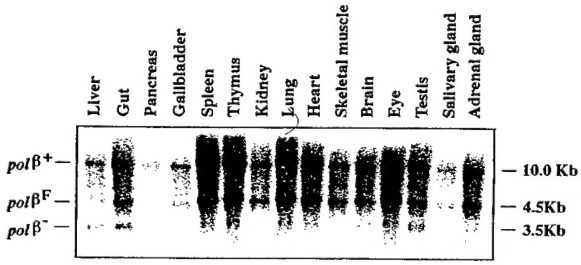

Figure 2C. Southern blot analysis of various tissues from a TTR10-3 mouse carrying polβ$^{F/+}$ alleles. Ten micrograms of genomic DNA from each tissue was digested with BamHI and subjected to Southern blot analysis using the polβ probe. Cre-dependent deletion of the *loxP* sites was observed mainly in digestive organs such as liver, pancreas, gut, and gallbladder with an efficiency of 95%, 100%, 50%, and 50%, respectively.

Table 1. Summary of cDNA clones from RDA analysis of anti-IgM activated B cell (BL2) products

	Number of cDNA clones		GenBank Accession number	dbEST Id
Known sequences	24	Representing 11 genes		
	8	DEC1	NM_003670	
	3	EGR2	J04076	
	3	hIL6R	X12830	
	2	SLP76	NM_005565	
	2	homo B/K protein	AF220560	
	1	SIRPalpha	Y10375	
	1	MDC	U83171	
	1	A20	M59465	
	1	hnRNPA2	NM_002137	
	1	LFA1	M29487	
	1	BCAR3	NM_003567	
New Genes	6			
	3		AW755261	4215617
	1		AW755262	4215618
	2		AW755263	4215619

DEC1 refers to a transcription factor expressed in differentiated human embryo chrondrocytes, EGR2 is early growth response protein, hIL6R is IL6 receptor, SLP76 is SH2 domain containing Leukocyte Protein of 76 kDa, SIRP alpha is from a family of human proteins that inhibits signaling through tyrosine kinase receptors, MDC is human macrophage-derived chemokine precursor, and A20 is a human transcription factor important in apoptosis cell signaling, hnRNP is heterogeneous nuclear ribonucleoprotein, and LFA is Leukocyte adhesion glycoprotein, BCAR3 is a breast cancer anti-estrogen resistance 3 gene.

APPENDICES:

1) Key research accomplishments:
   a. Generation of liver-specific Cre transgenic mice.
   b. Generation and analysis of liver-specific STAT3 knockout mice
   c. Generation and analysis of endothelial cell-specific STAT3 knockout mice
   d. Generation and analysis of dendritic cell-specific STAT3 knockout mice
   e. Establishment of representational difference analysis (RDA) and analysis of gene regulation in B-cells using RDA
   f. Analysis of STAT activation in NIH3T3 cells transformed by receptor tyrosine kinases

2) Reportable outcomes:
   i) Manuscripts published:

      a. Nakayama, Y., Iwamoto, Y., Maher, S.E., Tanaka, Y. and Bothwell, A.L.M. (2000) Altered gene expression upon BCR cross-linking in Burkitt's lymphoma B cell line. ***Biochem. Biophys. Res. Commun.***, 277, 124-127.

   ii) Developed reagents:
      a. Liver-specific Cre transgenic mice
      b. Digestive organ-specific Cre transgenic mice
      c. NIH3T3 transformants with STAT3 mutants and erbB receptors.

   iii) Funding applied for based on work supported by this award:

      a. Gustavus and Louise Pfeiffer Research Foundation Award
      b. Medical College of Wisconsin Cancer Center Interdisciplinary Grant

   iv) Employment received based on experiences/training supported by this award:

      a. Assistant Professor in the Division of Urology / Human Molecular Genetics Center at the Medical College of Wisconsin

3) Attached are an original and two copies of the above cited manuscript.

Altered Gene Expression upon BCR Cross-linking in Burkitt's Lymphoma B Cell Line

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Abbreviations used: GC, germinal center; FDC, follicular dendritic cell; DC, dendritic cell; BCR, B cell receptor; RDA, Representational Difference Analysis; SIRP, signal-regulatory protein; SH2, src homology 2; SLP76, SH2 domain containing leukocyte protein of 76 kDa; MDC, macrophage derived chemokine; CD40L, CD40 Ligand; CCR4, CC chemokine receptor 4; BCAR3, breast cancer anti-estrogen resistance 3; LFA, lymphocyte function-associated antigen; IL6R, interleukin 6 receptor; EGR2, early growth response protein; TCR, T cell receptor.

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Abstract:
Burkitt's lymphoma cell line, BL2 was stimulated by surface BCR cross-linking and altered gene expression was analyzed by RDA methodology. Consistent with previous reports, we detected up-regulated MDC, IL6R and adhesion molecule LFA1. We also detected gene expression of SIRPa, anti-apoptotic A-20, signal regulatory SLP76 and BCAR3, DNA binding proteins EGR2 and DEC1 in addition to some new genes.
Key words:
Burkitt's lymphoma cell, BCR cross-linking, RDA, gene expression, transcription factors.

INTRODUCTION:
When B cells encounter T cell dependent antigen, the antigen reactive but still low affinity antibody producing B cells migrate into lymph nodes and spleen. The activated B cells form germinal centers (GC) and proliferate at a high rate accompanying isotype switching and hypermutation in their immunoglobulin genes. Affinity maturation takes place in the GC microenvironment. Formation of GC provides a unique microenvironment in secondary lymphoid organs which promotes interactions between B cells and surrounding T cells, Follicular Dendritic Cells (FDC) and Dendritic Cells (DC). Antigen specific B cells not only undergo signaling from surrounding cells but also actively participate in these multiple and complex interactions (1).

Surface B cell receptor (BCR) cross-linking results from interaction with antigens. However, BCR cross-linking alone could induce apoptosis or arrest of proliferation of B cells. B cell apoptosis induced by BCR cross-linking can be rescued by co-activation of CD40 by CD40 Ligand (CD40L) on T cell. T cell contact stimulation on B cell provides

various signals through cell surface molecules including CD40, B7, MHC class II on B cell by CD40L, CD28, CTLA-4 and TCR on T cell.

The Burkitt's lymphoma B cells are considered as B cells isolated at a point of differentiation by transformation (2). The Burkitt's lymphoma cell line, BL2, shows cell surface marker proteins characteristics of centroblastic B cell phenotypes which suggests it is of centroblastic B cell origin (3). BCR activation is a particularly important step for late stages of differentiation of B cell. In this paper, we characterized altered gene expression in BL2 cells by BCR cross-linking as a model study of antigen stimulated germinal center centroblastic B cells by applying PCR-coupled cDNA Representational Difference Analysis (RDA). Our results may identify important signaling pathway that exist in complex B, T, FDC, DC networks in the microenvironment of secondary lymphoid organs.

Materials and Methods:
Cells: Burkitt's lymphoma B cell line, BL2, was generously provided by Dr George Miller at Yale Medical School. The cell line was cultured in 10% FCS containing complete RPMI1640 medium at 37C.

Antibody cross-linking of BL2 cells: BL2 cells were incubated at a density of 0.5 x 106 cells/ml in complete RPMI 1640 medium containing 10 mg /ml anti-human IgM goat IgG F(ab')2 (Jackson ImmunoResearch Lab.Inc., West Grove, PA) for thirty six hours prior to harvest for RNA preparations. At this concentration of anti-IgM treatment, BL2 growth was arrested after doubling their cell numbers. The anti-IgM stimulated and non-stimulated cells were processed at the same time to minimize experimental variation. Cells were resuspended in 4 ml PBS, and immediately loaded on 3 ml Ficoll/Paque (Pharmacia, Uppsala, Sweden) and centrifuged at 4000 rpm for 20 min at RT. Live cells were collected and washed with cold PBS for three times and RNA isolated.

RNA and cDNA preparations and RDA: RNA extraction was carried out by Trizol (Gibco/BRL, Grand Island, NY). About 1.0 x106 cells were extracted with 0.5 ml of Trizol at RT for 5 min followed by 100 ml chloroform extraction. The RNA was precipitated by adding 5 mg glycogen and 300 ml isopropanol and spun at 12,000 for 20 min at 5C. After removal of residual genomic DNA from total RNA by DNAse I (RNase free) (Boehringer Mannheim, Indianapolis, IN) followed by phenol and chloroform extractions, mRNA was prepared using an Oligotex mRNA isolation kit (Qiagen, Valencia, CA). Elution was carried out with 6 ml of 75C preheated elution buffer twice. The remaining steps for RDA were performed as described by Hubank and Schatz (1994). The final third differential products were cut with DpnII and DNAs were subcloned into the BamHI site in the pBlueScript II SK vector. DNA sequences of individual clones were determined with ABI PRISM 373A DNA sequencer (PE Biosystems, Foster City) using Thermo Sequenase kit (Amersham, Piscataway, NJ) with T7 and Reverse sequencing primers.

Results and Discussion:
RDA technology is a powerful and efficient technology using PCR amplification with specific linkers as primers combined with subtraction of product of control cells from product of test cells that allows detection of differentially expressed genes (4-7). A summary of 30 clones identified by RDA analysis of activated BL2 cells is presented in Table 1. We detected gene expression of MDC, IL6R as well

as SIRPa, adhesion molecule LFA1, anti-apoptotic A-20, signal regulatory SLP76 and BCAR3, DNA binding proteins EGR2 and DEC1 in BCR cross-linked Burkitt's Lymphoma BL2 cell. It is known that MDC in unstimulated B cell lineage (8) and IL6R in BL2 cell line are not expressed (9,10). LFA1 molecule is significantly up-regulated upon B cell stimulation with anti-IgM and IL4 (11). Consistent with previous observations, we detected expression of MDC, IL6R and LFA1 in the BL2 cell stimulated by BCR cross-linking.

MDC is a recently identified member of the CC chemokine family and attracts T cells and expression is detected in macrophage, monocyte derived DC and NK. MDC interacts with CC chemokine receptor 4 (CCR4). Only in CCR4-transfected cells, but not other CCR family member receptor transfectants, a calcium flux was induced by MDC (12). CCR4 chemokine receptor is expressed on the surface of T cells, thus, MDC can provide a recognition signal to T cell (13). MDC was expressed in maturing DC. During maturation of DC, MDC expression is up-regulated in vivo in lymph nodes as well as in vitro (14,15). Such maturing DC, supposedly due to MDC, preferentially attract antigen activated T cells more strongly than naive T cells and are thought to promote DC encounter with antigen-specific T cells (14). On the other hand, in murine and human B cells no mRNA expression is detected in unstimulated cells (8). In B cells, so far MDC was detected after CD40 stimulation (8). However, for T cell to be able to stimulate B cell through CD40, already established T-B cell contact is a prerequisite because CD40L is expressed on T cell. We detected induced MDC by BCR cross-linking in BL2 cell. In GC environment perhaps MDC production by antigen stimulated B cells could recruit antigen activated T cell to facilitate T cell help. Upon antigen stimulation apoptosis could be induced in B cell. Activated B cell could be rescued from apoptosis by CD40 stimulation by CD40L on recruited activated T cell.

It is known that CD40 activation can induce A20 in B cell (16). However, we observed expression of anti-apoptotic A-20 gene in the B cell by BCR cross-linking. In BCR activated B cells, A-20 induction may enhance resistance to apoptosis.

We detected three src homology 2 (SH2) domain related signal molecules. Signal-regulatory proteins (SIRP) are transmembrane glycoproteins with three extracellular Ig like domains (17). Previously it has been reported that SIRP is selectively expressed by myeloid cells (macrophages, monocytes, granulocytes, DCs) and neurons (17). No SIRP expression was detected in T-cell or B-cell lines (18). A member of SIRPa family expressed on monocytes and a sub-population of dendritic cells mediate binding to CD4 T cells (19). The SIRP cytoplasmic tail contains two immunoreceptor tyrosine-based inhibition motifs (ITIM) and is a substrate of activated protein tyrosine kinases (PTK), and its tyrosine-phosphorylated form binds SHP-2 through its SH2 domain. It also binds SHP-1 and Grb2 in vitro (20). SIRPs negatively regulate signaling through PTKs, however, their physiologic functions are not well characterized (17,18). We speculate that SIRPa expressed in B cell could play important roles in B cell as a negative regulator and also could mediate interaction and signaling with CD4 T cells.

We detected expression of intracellular signaling molecules SLP76 (SH2 domain containing Leukocyte Protein of 76 kDa) in the BCR stimulated B cells. SLP76 is a crucial component in T cell. Previously, in spleen

and in B cell lines SLP76 expression was detected in addition to in peripheral blood leukocytes, thymus and T and monocyte cell lines (21). SLP76 can interact with Vav and cooperate to induce activity of the transcription factor NF-AT and IL-2 expression. SLP-76-deficient mice exhibit a profound block in T-cell development (22). SLP-76 is a substrate of the TCR activated protein tyrosine kinase pathway which associates with the adaptor protein Grb2. SLP76 could also be involved with signal regulation by SIRP. The role of SLP76 in signal transduction in B cell has not been characterized, however, it could play an important role in B cell signaling.

We also detected a recently reported breast cancer anti-estrogen resistance 3 (BCAR3) which has an SH2 domain as well as CDC48 homologous domain in its c-terminus and is involved in anti-estrogen resistance in human breast cancer cells (23). BCAR3 could be involved in signal transduction and could have important rolls in B cell.

It is interesting that we detected IL6R in BL2 cell by BCR cross-linking. IL6 together with IL2 stimulate B cell differentiation to an antibody secreting cell. Both naive and memory B cells can be induced to secrete IL-6 upon CD40 stimulation while in GC B cell stage there is no IL6 (1). On the other hand, its receptor, IL6R, is inducible at a certain stage. Induction of gp80 allows formation of an IL6R heterodimer on the cell surface together with constitutively expressed gp130 that allows IL6 responsiveness of the B cell (1,24,25). IL6R expression could be up-regulated upon BCR stimulation as well as previously reported anti CD40 stimulation. Thus, it is a functional characteristic of human GC B lymphocytes to lose the ability to induce IL-6 and to acquire the ability to induce IL6R in GC is parallel. This may permit better control of B cell growth and differentiation to develop to antibody secreting cells during the germinal center reaction (1).

It is noteworthy that the expression of recently identified transcription factors, EGR2 and DEC1 were also detected in the BCR stimulated BL2 cells. EGR2 was first identified as an immediate-early response gene, encoding a Cys2His2 type Zinc finger protein that binds DNA in a sequence-specific manner and acts as a transcription factor and may play a role in the regulation of cellular proliferation (26). Egr2 knockout mice display hypomyelination of the peripheral nervous system and a block of Schwann cells at an early stage of differentiation (27). DEC1 was cloned from Bt2cAMP stimulated human chondrocytes. Its predicted sequence has a basic helix-loop-helix (bHLH) which is found in the mammalian HES family, Drosophila hairy, and Enhancer of split m7. DEC1 was expressed in various tissues including the cartilage, lung, spleen, and intestine, but not in the brain (28). These genes of DNA binding protein are recently identified and functions are currently not well understood. Because of their significant DNA binding motifs and induction by BCR cross-linking, these genes may have significant physiological roles in B cell signaling.

In addition, we obtained three new sequences by RDA. Their GenBank EST accession numbers are listed in Table 1. The AW755261 matched with other ESTs obtained from tonsil (AI567785) and lymph (AI433612). Among 500bp of AW755261, 50bp has 86% homology to human proto-oncogene tyrosine-protein kinase (abl) gene (GenBank accession number,

U07563.1). It is said that abl gene is the cellular homolog proto-oncogene of Abelson's murine leukemia virus and is associated with t9:22 chromosomal translocation with the BCR gene in chronic myelogenous and acute lymphoblastic leukemia.

BCR stimulation is important for late stages of B cell differentiation. These expressed genes with already known as well as unknown sequences in BCR crosslinked B cell could be potentially involved in B cell differentiation, development and hypermutation processes. The genes detected in BL2 model in this study are from a Burkitt's lymphoma cell. Currently analysis in tissue and stage specific expression of the known genes discussed here as well as cloning the full length cDNA of the unidentified genes are in progress.

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Table 1. Summary of cDNA clones from RDA analysis of anti-IgM activated B cell (BL2) products

	Number of cDNA clones		GenBank	dbEST Id
Known sequences	24	Representing 11 genes	Accession number	
	8	DEC1	NM_003670	
	3	EGR2	J04076	
	3	hIL6R	X12830	
	2	SLP76	NM_005565	
	2	homo B/K protein	AF220560	
	1	SIRPalpha	Y10375	
	1	MDC	U83171	
	1	A20	M59465	
	1	hnRNPA2	NM_002137	
	1	LFA1	M29487	
	1	BCAR3	NM_003567	
New Genes	6			
	3		AW755261	4215617
	1		AW755262	4215618
	2		AW755263	4215619

DEC1 refers to a transcription factor expressed in differentiated human embryo chrondrocytes, EGR2 is early growth response protein, hIL6R is IL6 receptor, SLP76 is SH2 domain containing Leukocyte Protein of 76 kDa, SIRP alpha is from a family of human proteins that inhibits signaling through tyrosine kinase receptors, MDC is human macrophage-derived chemokine precursor, and A20 is a human transcription factor important in apoptosis cell signaling, hnRNP is heterogeneous nuclear ribonucleoprotein, and LFA is Leukocyte adhesion glycoprotein, BCAR3 is a breast cancer anti-estrogen resistance 3 gene.